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EXAMINER	
DUNSTON, JENNIFER ANN	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 01/12/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/822,760	MIYOSHI ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 October 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1 and 5-14* is/are pending in the application.

4a) Of the above claim(s) *5-14* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *17 October 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

This action is in response to the amendment, filed 10/17/2005, in which claims 2-4 were canceled, and claim 1 was amended. Applicants' arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections and objections not reiterated in this action have been withdrawn. **This action is FINAL.**

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Election/Restrictions

Claims 5-14 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 3/11/2005.

This application contains claims 5-14 drawn to an invention nonelected with traverse in the reply filed on 3/11/2005. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Claim 1 reads on elected Group I and, as a result of the amendment filed 10/17/2005, is no longer generic to Groups II and III.

Drawings

The drawings were received on 10/17/2005. These drawings are accepted.

Claim Rejections - 35 USC § 112

Claim 1 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. **This rejection has been altered to address the amendment to the claims, filed 10/17/2005.**

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention: The claim is drawn to a method for identifying a pluripotent hepatic progenitor cell, comprising detecting the presence of a sugar chain indicative of a pluripotent hepatic progenitor cell using a kidney bean lectin or a lentil lectin. The claim defines a pluripotent hepatic progenitor cell as a cell capable of differentiating into bile duct cells or hepatocytes.

The nature of the invention is complex in that the detection of a sugar chain expressed on the pluripotent hepatic progenitor cell must be sufficient to identify the cell as a pluripotent hepatic progenitor cell. The claims do not limit the population of cells from which the pluripotent hepatic progenitor cell can be identified. Thus, the method encompasses embodiments where the hepatic progenitor cell is identified in a mixed population cells from the liver, bone marrow or peripheral blood, for example. Further, the invention is complex due to the type of cell identified by the claimed method. The specification does not provide a clear

definition as to what qualifies a cell as being a pluripotent hepatic progenitor cell; however, it is noted in the instant specification that "an oval cell, a hepatoblast, a marrow-derived cell and the like have been considered as a candidate for a stem cell in the liver" (paragraph [0004]). As stated in the instant specification, "it is current circumstance that a stem cell in the liver is not clearly defined and that the above-mentioned candidate cells for a hepatic stem cell are merely found" (paragraph [0005]). In addition, it is current circumstance that what cell surface antigen is expressed at each stage of differentiation from a stem cell to a mature cell has not been sufficiently analyzed." Therefore, the identification of a pluripotent stem cell by detecting a sugar chain expressed on the pluripotent hepatic progenitor cell is a complex and unpredictable process (see the discussion under *Predictability and state of the art* below). Further, the binding of lectins to sugars on the surface of cells is complicated by the surface charge, anomeric sugar linkage, location of the specific sugar residue in the complex carbohydrate chain, and its position within the conformational structure of the protein core (McMillan et al. J. Histochem. Cytochem. Vol. 36, No. 12, pages 1561-1571, 1988; e.g. paragraph bridging pages 1568-1569). Moreover, the lectins recited in claim 1 each have different specificities with regard to detecting sugar chains (e.g. instant specification, paragraph [0028]), which adds to the complexity of the invention.

Breadth of the claims: The claims are limited to the use of kidney bean lectin or lentil lectin to identify a pluripotent hepatic progenitor cell.

Guidance of the specification and existence of working examples: The specification states the following with regard to the basis of the invention at hand:

> The present invention is based on the surprising finding found by the present inventors that a pluripotent hepatic progenitor cell, specifically, a glycoprotein on

> the cell surface has specifically high reactivity with kidney bean lectin lE4PHAI, wheat germ lectin (WGA), lentil lectin (LCA), or Aleuria aurantia lectin (AAL), and is significantly different from a glycoprotein from a primary culture hepatocyte or a hepatic cancer cell. Further, the present invention is based on the surprising finding by the present inventors that a pluripotent hepatic progenitor cell can be identified by using the above-mentioned glycoprotein, specifically, a cell surface sugar chain antigen as a marker, and that the cell can be sorted using cell separation technique such as flow cytometry. See paragraph [0014].

The specification envisions the identification of hepatic progenitor cells from a cell population containing various cells at high specificity by using the sugar chain expressed on the cell surface (e.g. paragraph [0017]). Further, the specification envisions identifying pluripotent hepatic progenitor cells in a process comprising (i) contacting any organ, tissue or the like, without any particular limitation, as long as the material is expected to contain the pluripotent hepatic progenitor cell and includes, for instance, an adult liver tissue, a fetal liver tissue, a bone marrow tissue, a blood cell, a marrow cell, a peripheral blood cell, and the like, with a protein capable of binding to the sugar chain expressed on the pluripotent hepatic progenitor cell, (ii) detecting a cell with the protein bound thereto, in the mixture obtained in step (i) (e.g. paragraphs [0021], [0022], [0025]). The specification envisions the use of any protein capable of binding to, or associating with, the sugar chain for the detection of the sugar expressed on the surface of the pluripotent hepatic progenitor cell (e.g. paragraph [0027]). The specification envisions the use of lectins, which are sugar-binding proteins or a glycoprotein other than an imunoreaction product, which can aggregate cells or a complex carbohydrate [e.g. paragraph [0027]). Further, the specification teaches the preferred use of a lectin capable of reacting with a sugar chain specifically expressed on the cell (e.g. paragraph [0027]). The specification suggests that lectins capable of reacting with sugar chains specifically

expressed on pluripotent hepatic progenitor cells include those capable of binding L-fucose, D-galactose, N-acetyl-D-galactosamine, D-mannose, di-N-acetylchitobiose, sialic acid, etc. (e.g. paragraph [0027]). The specification envisions the use of kidney bean lectin, which binds to a complex-type sugar chain structure having a bisecting GlcNAc structure; wheat germ lectin, which binds a hybrid-type or complex-type sugar chain structure having a sialic acid and/or bisecting GlcNAc structure; lentil lectin, which binds duplex complex-type sugar chain of a structure in which α-L-fucose residue is bound to N-acetylglucosamine residue positioned at a reducing end, or a triplex complex-type sugar chain of a branch at C-2,6 of α-D-mannose residue, etc. (e.g. paragraph [0028]). The specification teaches the use of different methods to identify lectin binding to the cell, including lectin blotting method, lectin column method, lectin staining method with labeled lectin, and flow cytometry using a labeled lectin (e.g. paragraph [0030], [0031], [0034], [0035]). The specification also envisions the detection of sugar chains on the surface of the pluripotent hepatic progenitor cells using an antibody, or by indirectly testing for the presence of a sugar by measuring the expression of an enzyme involved in the synthesis of the sugar chain, such as N-acetylglucosaminyltransferase III (GnT-III), for example (e.g. paragraph [0036] and [0045]).

The working examples test the binding of lectins to the glycoproteins expressed by a rat epithelial (RLE) cell line as compared to control cells, including human hepatoblastoma cell lines, a rat hepatic cancer cell line, a mouse fetal hepatocyte cell line, and a mouse fetal hepatic cancer cell line (e.g. paragraph [0061]). In Example 1, the binding of kidney bean lectin (E4PHA) and wheat germ lectin (WGA) was tested using a

lectin blotting assay. Although the results demonstrate that the RLE cell was very strongly stained with E4PHA as compared to the hepatoblastoma cells, hepatic cancer cells or fetal hepatocytes, the presence of binding to cells other than E4PHA rules out specific binding of E4PHA to hepatic progenitor cells. In Example 2, the binding of lentil lectin (LCA), E4PHA, and Concanvalin A (ConA) was testing using a flow cytometry assay. Although the results demonstrate that RLE cells were more strongly stained with E4PHA and LCA as compared to control cells, these results do not demonstrate the specificity of lectin binding for pluripotent hepatic progenitor cells. In fact, hepatic non-parenchymal cells and hepatic cancer cells are bound by WGA (e.g. paragraph [0074]). Further, the working examples teach that the RLE cell bound by E4PHA was capable of differentiating into a bile ductal cell or a hepatocyte, demonstrating that the RLE cell is capable of pluripotent differentiation (e.g. paragraphs [0076]-[0078]). Example 4 teaches the presence of GnT-III expression RLE cells but not m31 cells or hepatocytes.

The specification does not provide guidance with regard to sugar chains that are specifically expressed on the surface of hepatic progenitor cells. Thus, the specification fails to provide guidance with regard to the lectins, or any other protein, that can be used to identify pluripotent hepatic progenitor cells in a mixed population of cells. As noted in the working examples, the tested lectins (e.g. kidney bean lectin and wheat germ lectin) are not specific to hepatic progenitor cells in that they detect sugars expressed in other cells such as hepatic cells and hepatic cancer cells. Further, the absence of a sugar chain on one or two cell types does not rule out the possibility that the sugar chain expressed on

the RLE cell line is not similarly expressed on another cell type obtained from the liver, bone marrow or peripheral blood. Furthermore, the RLE cell line is the only cell line used to determine lectin binding and thus the only organism tested for lectin binding is the rat. The specification does not address potential species differences in sugar chain expression. Therefore, the specification does not teach the identification of a pluripotent hepatic progenitor cell from any tissue or organ by detecting a sugar chain expressed on the cell surface.

Predictability and state of the art: The nature of the invention is unpredictable for a number of reasons: (i) pluripotent hepatic progenitor cells are not a well-defined population of cells, (ii) the specificity of a sugar chain expression on one cell type relative to others cannot be predicted and must be determined experimentally.

As noted in the instant specification, the stem cells of the liver are not clearly defined and cells such as oval cells are merely candidate hepatic progenitor cells (e.g. paragraph [0004]-[0005]). Populations of oval cells are known to constitute a heterogeneous cell compartment containing cells that may differ in their differentiation capacity and stage of differentiation (Fausto, Hepatology, Vol. 39, No. 6, pages 1477-1487, 2004; e.g. page 1478, right column, 1st full paragraph). Furthermore, Fausto notes that the presence of oval cells or hepatic stem cells in the bone marrow is controversial in the art in that some studies have demonstrated that bone marrow cells were not the source of oval cells that were capable of repopulating livers after liver injury (e.g. pages 1478-1479, Relationships Between Oval Cells and Hematopoietic Stem Cells; pages 1482-1483, Generation of Hepatocytes by Bone Marrow Cells and Cell Chimerism in

Recipients of Liver and Bone Marrow Transplants). Thus, the complex nature of the invention is further compounded by a lack of a clear definition of a pluripotent hepatic progenitor cell.

An analysis of the prior art as of the effective filing date of the present application identified teachings with regard to testing cells for sugar chain expression. For example, McMillan et al (J. Histochem. Cytochem. Vol. 36, No. 12, pages 1561-1571, 1988) teach that wheat germ lectin (WGA), lentil lectin (LCA), and kidney bean lectin (PHA) all bind to hepatocytes (e.g. Table 1; page 1563). Thus, the prior art teaches that these lectins are not specific for pluripotent hepatic progenitor cells. Further, lectins such as *Ricinus communis* agglutinin (RCA I), *Concanavalia enisformis* agglutinin (ConA) and *Pisum sativum* agglutinin PSA bind hepatocytes (e.g. McMillan et al, Table 1). Rambhatla et al (US Patent Application Publication No. 2002/0160511) teach that lectin liver progenitors and biliary epithelium are positive for lectin binding (e.g. paragraph [0112]; Table 1). Moreover, Makino et al (Gastroenterologia Japonica, Vol. 23, No. 6, pages 658-665, 1988) characterize oval cells, putative pluripotent hepatic progenitor cells, with seven biotinylated lectins (e.g. Table 1). Oval cells were observed in rats treated with α-naphthylisothiocyanate (ANIT), 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) and 2-acetylaminofluorene (2-AAF) (e.g. page 659, Materials and Methods; page 660, Histological Findings). However, the oval cells did not have a consistent pattern of sugar chain expression; those obtained from 2-AAF and 3'-Me-DAB treatment showed increased binding to peanut agglutinin (PNA), whereas oval cells induced by ANIT showed an increased binding of *Ulex europaeus* agglutinin I (UEA I) (e.g. page 661, Discussion). Further, Makino et al teach that WGA detects sugars on normal bile duct as well as cells in the biliary obstruction rat, and ANIT, 2-AAF, or 3'-Me-DAB treated rat (e.g. Table 2).

Species differences in glycosylation of membrane bound proteins will result in species differences in sugar chain expression on the surface of cells. For example, Lascols et al (Cell Mol Biol (Noisy-le-grand). Vol. 40, pages 359-71, 1994) teach that the prolactin receptor of mouse and rat have different glycosylation patterns (e.g. Table 2; Figure 3; pages 368-370, Discussion). The additive effects of a number of species differences in glycosylation patterns may result in different patterns of sugar chain expression. Thus the results obtained for a cell line from one species will not necessarily apply to cells of other species.

Moreover, detection of sugar chain expression with a single lectin may not sufficiently describe a cell such that it can be identified as a pluripotent hepatic progenitor cell. Irimura et al (US Patent Application Publication No: 2004/0091938) teach that an individual lectin cannot give microscopic information for appropriately reflecting the variety of sugar chains on a cell (e.g. paragraphs [0002]-[0003]). However, Irimura et al note that a lectin sub-library comprising a combination of different types of lectins can be used as a tool to identify and to make a comparison of sugar chains wherein a difference in cells is represented by a difference in sugar chains that can be discriminated [0004]).

Therefore, the prior art teaches that cells other than pluripotent hepatic progenitor cells express sugars on their cell surface, and these sugars are shared between pluripotent hepatic progenitors and other cell types. Thus, the detection of sugar chain expression by any means such as lectin binding, antibody binding or detecting the expression of an enzyme involved in sugar chain synthesis will be insufficient to identify a pluripotent hepatic progenitor cell.

Amount of experimentation necessary: Given the lack of guidance in the specification and prior art with regard to the detection of a sugar chain expressed on a cell for the purpose of

identifying the cell as a pluripotent hepatic progenitor cell, the quantity of experimentation in this area is very large. Clearly, the prior art teaches that hepatic progenitor cells are not the only cells that express sugar chains on the cell surface. Further, the prior art teaches that lectins such as WGA, LCA and PHA are able to detect sugar chains on cells other than pluripotent hepatic progenitor cells. Thus, the skilled artisan would have to conduct a large number of trial and error experiments to learn which sugars, if any, could be used to identify a pluripotent hepatic progenitor cell. The skilled artisan would need to amass a large number of different reagents capable of detecting sugar chains expressed on the cell surface, such as a panel of lectins, for example. Next, the skilled artisan would have to test the panel on putative pluripotent hepatic progenitor cells of multiple different species, and compare the results to the other cell types of the same species to identify sugar chain expression specific to pluripotent hepatic progenitor cells. If no sugar chain were specifically expressed only on pluripotent hepatic progenitor cells, which is likely given the unpredictability of the art, one would have to determine if the detection of a particular combination of sugar chains is specific to pluripotent hepatic progenitor cells. If a combination specific to hepatic progenitor cells cannot be identified, one would have to quantify the level of sugar expression and determine threshold values for the identification of pluripotent hepatic progenitor cells versus all other cells of the body. Moreover, one would have to conduct a number of experiments to validate that the cell identified by the method is a pluripotent hepatic progenitor cell capable of differentiating into multiple different cell types or tissues, including hepatocytes. This would require a large amount of inventive effort, with each of the many intervening steps, upon effective reduction to practice, not providing any guarantee of success in the succeeding steps.

In view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art, the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention. Therefore, claim 1 is not considered to be enabled by the instant specification.

Response to Arguments - 35 USC § 112

The rejection of claims 1-4 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, has been withdrawn in view of Applicant's amendment.

Applicant's arguments filed 10/17/2005 have been fully considered but they are not persuasive. The response asserts that claim 1 is fully enabled by the specification (see page 8 of the response). The response points to Examples 1 and 2 of the specification as an evidentiary basis for the response.

The working examples test the binding of lectins to the glycoproteins expressed by a rat epithelial (RLE) cell line as compared to control cells, including human hepatoblastoma cell lines, a rat hepatic cancer cell line, a mouse fetal hepatocyte cell line, and a mouse fetal hepatic cancer cell line (e.g. paragraph [0061]). In Example 1, the binding of kidney bean lectin (E4PHA) and wheat germ lectin (WGA) was tested using a lectin blotting assay. Although the results demonstrate that the RLE cell was very strongly stained with E4PHA as compared to the hepatoblastoma cells, hepatic cancer cells or fetal hepatocytes, the presence of binding to cells other than E4PHA rules out specific binding of E4PHA to hepatic progenitor cells. In Example

2, the binding of lentil lectin (LCA), E4PHA, and Concanvalin A (ConA) was testing using a flow cytometry assay.

Although the results demonstrate that RLE cells were more strongly stained with E4PHA and LCA as compared to control cells, these results do not demonstrate the specificity of lectin binding for pluripotent hepatic progenitor cells in general. For example, McMillan et al (J. Histochem. Cytochem. Vol. 36, No. 12, pages 1561-1571, 1988) teach that wheat germ lectin (WGA), lentil lectin (LCA), and kidney bean lectin (PHA) all bind to hepatocytes (e.g. Table 1; page 1563). Thus, the prior art teaches that these lectins are not specific for pluripotent hepatic progenitor cells. Although the working examples teach assays that may be used to differentiate between RLE cells and some cell lines (human hepatoblastoma cell lines, a rat hepatic cancer cell line, a mouse fetal hepatocyte cell line, and a mouse fetal hepatic cancer cell line), the specification does not teach how to use kidney bean lectin or lentil lectin to identify a pluripotent hepatic progenitor cell from any cell population.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Response to Arguments - 35 USC § 102

The rejection of claims 1-4 under 35 U.S.C. 102(b) as being anticipated by Makino et al has been withdrawn in view of Applicant's amendment.

Conclusion

No claims are allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel can be reached at 571-272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR, http://pair-direct.uspto.gov) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your

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Jennifer Dunston
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jad

JAMES KETTER
PRIMARY EXAMINER